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Force Triggered Dissociation of the Highly Avid E9:Im9 Complex

David Brockwell[1], Oliver Farrance[1], Renata Kaminska[2], Sasha Derrington[1], Colin Kleanthous[2], Sheena Radford[1].
[1]University of Leeds, Leeds, United Kingdom, [2]University of Oxford, Oxford, United Kingdom.

Colicin E9 is a nuclease antibiotic produced by *E.coli* to target and kill competing bacteria in times of stress. Immunity protein 9 (Im9) is expressed co-translationally and binds strongly to E9, inactivating the nuclease to protect the producing cell from the cytotoxic effects of E9. The affinity between E9:Im9 is highly avid with an off-rate of the order of days. However, upon binding to an outer membrane protein of a competing cell, a complex set of protein:protein interactions coupled to protein remodelling results in rapid Im9 release allowing E9 translocation into the cytoplasm and cell death on a timescale of minutes.

In order to investigate the molecular basis for such an extreme switch in complex avidity we have used an atomic force microscope to perform dynamic force spectroscopy (DFS) measurements on E9:Im9. We observe a force-induced switching mechanism from the long lived complex (k_{off} ~ $1x10^{-5}$ s^{-1}) in the absence of force to a much shorter lived state (k_{off} ~ 5 s^{-1}) under the application of small biologically relevant forces. The decrease in complex lifetime under force can be abrogated by introduction of disulfide cross cross-links. This suggests that protein dynamics lie at the heart of the bipartite function of the complex switching from a highly stable complex protective to host, to one where rapid dissociation facilitates competitor death.

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The Persistent Effect of Initial Substrate Protein Conformation on Productive Folding by Groel-Groes

Jeremy S. Weaver, Hays S. Rye.
Texas A&M University, College Station, TX, USA.

The GroEL/GroES chaperonin system is required for the assisted folding of many essential proteins. In order to initiate a folding reaction, an open GroEL ring must capture a non-native substrate protein and encapsulate the protein inside an enclosed cavity that is formed when ATP and GroES bind to the same, substrate protein-occupied ring. Previous work demonstrated that the GroEL-dependent folding of highly recalcitrant proteins like RuBisCO is enhanced by two phases of structural disruption prior to the initiation of folding: a binding-driven unfolding phase upon substrate protein capture by a GroEL ring, and a forced expansion driven by the movement of the GroEL apical domains upon ATP binding. The overall impact, timing and significance of these unfolding events, particularly with regard to their coordination with GroES binding and substrate protein encapsulation, has remained controversial. Here we demonstrate that the conformation of a substrate protein at the moment of release into the GroEL-GroES cavity directly affects the efficiency of subsequent folding events. We first examined the conformation of non-native RuBisCO as it interacts with a GroEL variant possessing a substantially reduced ability to support folding. using a combination of resonance energy transfer and protease protection, we find that the non-native RuBisCO monomer adopts a less unfolded conformation upon initial capture by the GroEL variant. Subsequent binding of ATP results in a reduced forced expansion of RuBisCO by this GroEL variant. Despite efficient binding of GroES to a RuBisCO-occupied ring, however, the compaction of the folding intermediate during encapsulation is also substantially reduced. In combination, these deficits in initial structural alteration appear to result in a marked reduction in productive folding, suggesting a crucial role of these GroEL-dependent manipulations in preparation of the substrate protein for the folding process.

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Role of Nonspecific Interactions in Molecular Chaperones through Model-Based Bioinformatics

Andrew D. White[1], Wenjun Huang[2], Ann K. Nowinski[1], Shaoyi Jiang[1].
[1]University of Washington, Seattle, WA, USA, [2]University of Michigan, Ann Arbor, MI, USA.

Molecular chaperones are large proteins or protein complexes from which many proteins require assistance in order to fold. One unique property of molecular chaperones is the cavity they provide in which proteins fold. The interior surface residues which make up the cavities of molecular chaperone complexes from different organisms has recently been identified, including the well-studied GroEL-GroES chaperonin complex found in E. coli. It was found that the interior of these protein complexes is significantly different than other protein surfaces and that the residues found on the protein surface are able to resist protein adsorption when immobilized on a surface. Yet it remains unknown if these residues passively resist protein binding inside GroEL-GroEs, as demonstrated by experiments which created synthetic mimics of the interior cavity, or if the interior also actively stabilizes protein folding. To answer this question, we have extended entropic models of substrate protein folding inside GroEL-GroES to include interaction energies between substrate proteins and the GroEL-GroES chaperone complex. This model was tested on a set of 528 proteins and the results qualitatively match experimental observations. The interior residues were found to strongly discourage the exposure of any hydrophobic residues, providing an enhanced hydrophobic effect inside the cavity which actively influences protein folding. This work provides both a mechanism for active protein stabilization in GroEL-GroES and a model which matches current understanding the chaperone protein.

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Cooperative Motion of a Multi-Subunit Protein Visualized by X-Ray Single Molecule Tracking

Hiroshi Sekiguchi[1], Yohei Yamamoto[2], Ayumi Nakagawa[2], Kazuki Moriya[2], Mayuno Arita[2], Kouhei Ichiyanagi[3], Masafumi Yohda[2], Naoto Yagi[1], Yuji C. Sasaki[3].
[1]Japan Synchrotron Radiation Research Institute, Sayo, Japan, [2]Tokyo University of Agriculture and Technology, Koganei, Japan, [3]The University of Tokyo, Kashiwa, Japan.

Most of proteins or enzymes are multimeric proteins, such as dimer, trimer, or higher-order structures (Goodsell et al. Annu Rev Biophys Biomol Struct, 2000). Usually, these proteins have multiple biding sites for ligands, such as ATP, peptide and others, that induce protein's conformational change. The function of the protein is regulated by such conformational changes that may give rise to cooperativity. Therefore, assessment of the cooperativity between subunits of proteins is important to understand the function of complex proteins. We have previously reported that the diffracted X-ray tracking (DXT) method could trace ATP-induced twisting motion of group II chaperonin ring at a single molecule level with high accuracy. In DXT, a gold nanocrystal immobilized on one side of chaperonin-ring is used as tracer for structural change of chaperonin. Our analyses clearly showed that the chaperonin ring partially closed within 1 s of ATP binding, the closed ring subsequently twisted counterclockwise within 2-6 s, as viewed from the top to bottom of the chaperonin, and the twisted ring reverted to the original open-state with a clockwise motion. In this study, we checked how ATPase deficient mutant subunits modulate the speed or frequency of twisting motion. We controlled the number of ATPase deficient mutant within one chaperonin ring, constructed the ATP deficient hetero-ring using circular permutated connected mutants, and evaluated the effects of those mutants to chaperonin-ring's twisting motion. We found that the equivalent twisting motion was observed in hetero-ring chaperonin and inter-ring communication is dispensable for the function of group II chaperonin3.

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Precise Timing of ATPase Activation Drives Targeting of Tail-Anchored Proteins

Meera Rao, Michael E. Rome, William M. Clemons, Shu-ou Shan.
California Institute of Technology, Pasadena, CA, USA.

Hundreds of proteins are anchored on cellular membranes by a transmembrane domain (TM) at their extreme C-terminus. These 'tail-anchored' (TA) proteins perform numerous essential cellular functions, yet their unique topology poses fundamental challenges to their proper localization. In eukaryotic cells, the highly conserved ATPase Get3 coordinates the efficient capture and delivery of TA proteins to the endoplasmic reticulum (ER). How Get3 uses its ATPase cycles to drive this fundamental process remains elusive. Here we define the Get3 ATPase cycle and show that ATP specifically induces conformational changes in Get3 that culminate in its ATPase activation through tetramerization. This activation is inhibited by the TA protein loading complex Get4/5 and is activated by the TA substrate, ensuring the precise timing of ATP hydrolysis during targeting. Our results provide an explicit model for how Get3 harnesses the energy from ATP to spatially and temporally coordinate the membrane localization of TA proteins.